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The Association between the *MTR* Gene A2576G Polymorphism and Alzheimer's Disease: a Meta Analysis Study

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Abstract

Background: Alzheimer's disease (AD) individuals are characterized with high homocysteine (HCY) and low folate blood levels. Polymorphisms of genes encoding critical enzymes in folate metabolism have been associated with hyperhomocysteinemia and AD risk. An adenine to guanine transition at position 2756 (rs185087) of the methionine synthase (*MS* or *MTR*) gene causes hyperhomocysteinemia. However, the association between *MTR* A2756G polymorphism and AD remains controversial. We performed a Meta analysis pooling data from all relevant studies including cases and controls to reexamine the association between the *MTR* gene A2576G polymorphism and AD.

Methods: We applied random-effects or fixed-effects model according to the degree of heterogeneity to combine odds ratio (OR) and 95% coincidence intervals (95% CI). And we used the Quanto 1.2.4 software to calculate genetic power. Egger's test was carried out to evaluate the potential publication bias.

Results and discussion: Eight case-control studies enrolling 2,880 cases and 2,807 controls were included in this meta analysis. The overall ORs with 95% CIs showed no statistical association between the *MTR* gene A2756G polymorphism and the risk of AD in the allele contrast, the recessive model or dominant model for allele A (random-effects pooled OR 1.09, 95% CI 0.92-1.30; random-effects pooled OR 1.11, 95% CI 0.91-1.35; fixed-effects pooled OR 1.13, 95% CI 0.83-1.54, respectively). The genetic power was 11.6% in the recessive model and 43.7% in the dominant model. No association between *MTR* A2756G polymorphism and AD was observed, but the conclusion based on relatively small numbers of participants. Large heterogeneity was detected among combined populations in the contrast of AA *vs.* AG+GG ($p = 0.019$, $I^2 = 56.3\%$) and A *vs.* G ($p = 0.016$, $I^2 = 57.5\%$). One study was considered as the main cause of heterogeneity in both contrasts. The heterogeneity doesn't reduce in the subgroup analyses stratified by racial descents. It can be presumed that the heterogeneity mainly results from the diagnosis of AD and genotyping methods. No publication bias was observed.

Conclusions: In conclusion, the present Meta analysis suggests that *MTR* A2756G polymorphism is not a genetic determinant of AD. But small sample size may be one reason and it could not be ruled out that a true association exists.

Keywords: Alzheimer's disease; Single-nucleotide polymorphisms; Methionine synthase; Meta analysis

Background

Alzheimer's disease (AD) is the leading cause of dementia in the elderly, and its etiology is still not fully understood. Disease-causing mutations in the amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2) genes cause familial AD [1]. However, sporadic AD lacking an obvious familial aggregation accounts for as much as 90% patients of AD. Epigenetic modifications, such as DNA methylation, may contribute to the risk of sporadic AD [2]. Folate metabolism, also known as one-carbon metabolism, is required for the production of S-adenosylmethionine (SAM), which is the major DNA methylating agent [3].

Folate is essential nutrient required for one-carbon biosynthetic and epigenetic processes. Several investigators have measured plasma values of homocysteine (HCY) and folate in AD subjects. Overall, the majority of the studies agreed that plasma HCY values increased in AD subjects [4-6]; there was also indication that folate values reduced in the plasma of AD individuals respect to controls [4-7].

Polymorphisms of genes encoding critical enzymes in folate metabolism have been associated with hyperhomocysteinemia. Methionine synthase (*MS or MTR)* is a key enzyme in the one-carbon metabolism catalyzing HCY to methionine. An adenine to guanine transition at position 2756 (rs185087) of the *MTR* gene results in a substitution of aspartic acid for glycine and decreases methionine synthase activity. This polymorphism causes hyperhomocysteinemia [8]. However, results are still conflicting. Increased HCY levels have been reported in the presence of the wild type (*MTR* 2756A) allele [9], whereas other studies observed increased HCY levels in the presence of the mutant (*MTR* 2756G) allele [10,11].

Confused data were reported on the association between the *MTR* A2756G polymorphism and AD [12-21]. Some studies reported association between the *MTR* 2756AA genotype and AD [12,13,21]. But other studies revealed no association between the *MTR* A 2756G polymorphism and AD [14-20]. So we performed a Meta analysis of

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existing studies that examined allele and genotype frequencies of the *MTR* gene in patients with AD.

Methods

Search strategies

We searched MEDLINE (1966 to January 2012), EMBASE (1966 to January 2012), and Cochrane Collaboration Registry for Randomized Controlled Trials (1966 to January 2012). As a search criterion, we used the following: methionine synthase (*MS* or *MTR* gene) or *MTR* polymorphism and AD or Alzheimer's; or *MS* gene or *MS* polymorphism and AD or Alzheimer's. No language restriction was applied.

Selection criteria

We limited our search to full text, published articles and human studies. Abstracts, case reports, editorials, and review articles were excluded. We also retrieved relevant references of included studies for our search. When a report overlapped with a more detailed publication, only the latter was used. All studies that investigate the association of the *MTR* A2756G polymorphism with AD using a case-control design were considered in the meta analysis.

Clinical diagnosis of probable AD were all established according to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) [22], the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) working group criteria [23] and the Consortium to Establish a Registry for Alzheimer's disease (CERAD) working group criteria [24]. Controls were defined as subjects not meeting the dementia criteria with intact cognitive functions. All populations were consistent with Hardy-Weinberg equilibrium. Genotyping methods for each data set were described in the original publications.

Data abstraction

Two reviewers (Y. Wang and SL. Xu) independently extracted the data and disagreements were resolved by discussion. Characteristics abstracted from the studies included the name of first author, publication date, country origin, ethnicity, control characteristics, genotyping methods, total number of cases and controls, and numbers of cases and controls with *MTR* alleles and genotypes, respectively. Different ethnicity descents were categorised as Caucasian or Asian.

Quantitative data synthesis

The primary analysis was conducted by comparing the AA homozygous genotype with G-carrying genotypes, and also A allele with G allele. This meta analysis examined the contrasts of AA *vs* AG+GG and AA+AG *vs* GG, corresponding to the recessive and dominant effects, respectively of the A allele. We also examined the association between A allele and AD risk compared with that for G allele (A *vs* G). We used StataSE 12.0 statistical software packages to analyze our data. The odds ratio (OR) with 95% coincidence interval (95% CI) was calculated to assess the association of the *MTR* A2756G polymorphisms with AD risk.

We used Quanto 1.2.4 software to calculate genetic power [25] in the recessive model and dominant model for allele A, the high risk allele. The prevalence of AD in the general population was set as 0.4%, as reported in the Delphi consensus study in 2005 [26].

Heterogeneity between studies was assessed by using the chi-square-based Q-test and was considered statistically significant if $p < 0.1$[27]. Heterogeneity was quantified with the I^2 metric, which is determined by the formula (Q-df)/Q, where df is the number of degrees of freedom (1 less than the number of combined data sets). I^2 is considered large for values above 50%, ($I^2 < 25\%$: no heterogeneity; I^2= 25%–50%: moderate heterogeneity; I^2 = 50%-75%: large heterogeneity; I^2>75%: extreme heterogeneity) [28]. The pooled OR was calculated by the fixed-effects model (the Mantel-Haenszel method) when there was no or moderate heterogeneity among studies [29]. Otherwise, the random-effects model (the **DerSimonian-Laird** method) [30] was used. The Galbraith plot was used to spot the outliers as the possible major sources of heterogeneity [31].

Publication bias was assessed by visual inspection of Begger's funnel plots. Funnel plot asymmetry was assessed by the method of Egger's linear regression test, a linear regression approach to measure funnel plot asymmetry on the natural logarithm scale of the OR. The significance of the intercept was determined by the t-test ($p < 0.05$ was considered representative of statistically significant publication bias) [32].

Results and Discussion

Characteristics of included studies

The literature review identified nine articles for detailed assessment, one [20] of which was excluded because it was derived from the same study population as another report [12]. Our final analysis included eight case-control studies, enrolling 2,880 cases and 2,807 controls. Seven out of the eight studies involved Caucasian populations, and the other one was conducted in Asian population. Genomic DNA was extracted from blood samples in all the studies, and depending on the center, a broad range panel of technologies were used to genotype SNP. Detailed characteristics of the included studies are shown in Table 1, while both genotype and allele frequencies of AD patients and controls in the selected studies are showed in Table 2. The allele frequencies are calculated from the corresponding genotype distributions.

Meta analysis results

The overall OR with its 95% CI showed no statistical association between the *MTR* gene A2756G polymorphism and the risk of AD, as shown in Table 3.

The summary OR for AA *vs.* AG+GG was 1.11 by random-effects model (OR 1.11; 95% CI 0.91 to 1.35., Figure 1a). And the summary OR for AA+AG *vs.* GG was 1.13 by fixed-effects model (OR 1.13; 95% CI 0.83 to 1.54, Figure 1b). The OR for A *vs.* G is shown in Figure 1c. The summary OR with its 95% CI was 1.09 (0.92 to 1.30) by random-effects model.

In the stratified analysis by racial descent, no significant risks were found among Caucasians. The detailed data were shown in Table 3.

MTR is a key enzyme in the metabolism of HCY, catalyzing the remethylation of HCY to methionine. When the *MTR* reaction is impaired, as observed in vitamin B12 deficiency, a substantial proportion of cellular folate is converted into a metabolically unavailable form, which results in a functional folate deficiency [33]. *MTR* A2756G polymorphism was reported as a candidate gene polymorphism for coronary heart disease [34], cancer [35], and

Study	Country	Criteria	Genotyping methods	AD		Control	
				N (% Female)	Mean age	N (% Female)	Mean age
Beyer [12]	Spain	DSM-IV; NINCDS-ADRDA	RFLP	172(62%)	70.8	166 (60%)	68.7
Bosco [13]	Italy	CERAD	RFLP	152 (54%)	74.8	136 (55%)	69.3
Dorszewska [15]	Poland	NINCDS-ADRDA	RFLP	38(61%)	66.3 ± 12.2	50 (68%)	44.6 ± 16.2
Giedraitis [18]	Sweden (ULSAM)	NINCDS-ADRDA; DSM-IV	high and ultra-high throughput genotyping	86 (0%)	80.2(AAO)	404 (0%)	81.8
Li [17]	Canada	NINCDS-ADRDA	GWAS	753 (58%)	77.8 ±8.6	736 (64%)	73.4 ± 7.9
Linnebank [14]	Germany	DSM-IV	RFLP	162 (68%)	72 ±9	169 (56%)	71 ± 7
Reiman [16]	USA, Netherlands	NM	GWAS	861 (-)	74.9 ± 6.6	550 (-)	77.4 ± 7.3
Zhao [19]	China	DSM-IV; NINCDS-ADRDA	RFLP	353 (52%)	68.9 ± 9.2 (AAO)	346 (47%)	68.5 ± 9.1
Coppede [20]	Italy	DSM-IV; NINCDS-ADRDA	RFLP	375(63%)	74.2±6.46	307(63%)	71.7±8.02

Note: ULSAM, Uppsala Longitudinal Study of Adult Men; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders IV; NINCDS-ADRDA, the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association; CERAD, the Consortium to Establish a Registry for Alzheimer's Disease .NM, Not mentioned; RFLP, **restriction fragment length polymorphism;** GWAS, genome-wide association study; AAO, Age at onset

Table 1: Clinical characteristics of the populations included in the meta analysis.

Study	AD					Control				
	G-Allele	A-Allele	GG (frequency)	AG (frequency)	AA (frequency)	G–Allele	A-Allele	GG(frequency)	AG(frequency)	AA(frequency)
Beyer [12]	0.08	0.92	1 (0.006)	25 (0.145)	146 (0.849)	0.18	0.82	5 (0.030)	49 (0.295)	112 (0.675)
Bosco [13]	0.16	0.84	4 (0.026)	42 (0.276)	106 (0.697)	0.19	0.81	5 (0.036)	42 (0.307)	90 (0.657)
Dorszewska [15]	0.29	0.71	2 (0.053)	18 (0.474)	18 (0.474)	0.18	0.82	0 (0.000)	18 (0.360)	32 (0.640)
Giedraitis [18]	0.22	0.78	4 (0.047)	30 (0.353)	51 (0.600)	0.20	0.80	19 (0.048)	121 (0.303)	260 (0.650)
Li [17]	0.18	0.82	20 (0.029)	205 (0.297)	466 (0.674)	0.18	0.82	29 (0.043)	190 (0.279)	463 (0.679)
Linnebank [14]	0.22	0.78	7 (0.043)	58 (0.358)	97 (0.599)	0.26	0.74	8 (0.047)	71 (0.420)	90 (0.533)
Reiman [16]	0.19	0.81	29 (0.034)	259 (0.304)	563 (0.662)	0.19	0.81	20 (0.036)	167 (0.304)	363 (0.660)
Zhao [19]	0.07	0.93	2 (0.006)	47 (0.133)	305 (0.862)	0.08	0.92	2 (0.006)	54 (0.156)	290 (0.838)
Coppede [20]	0.14	0.86	12(0.032)	80(0.213)	283(0.746)	0.13	0.87	5(0.016)	72(0.236)	230(0.749)

Table 2: Distribution of *MTR* allele and genotype among AD cases and controls in the included studies.

Genetic contrasts	Population	Heterogeneity	Model used	OR (95% CI) p_{OR}	Egger's test
		p_h; I^2			t; p_E; (95% CI)
AA *vs.* AG+GG	Overall	0.019; 56.3%	random-effects (D-L)	1.11 (0.91-1.35) 0.305	0.54; 0.609 (-2.59-4.11)
	Caucasion	0.012; 61.1%	random-effects (D-L)	1.10 (0.88-1.37) 0.402	
AA+AG *vs.* GG	Overall	0.531; 0.0%	fixed-effects (M-H)	1.13 (0.83-1.54) 0.449	0.77; 0.464 (-5.08-1.00)
	Caucasion	0.425; 0.6%	fixed-effects (M-H)	1.13 (0.82-1.55) 0.445	
A *vs.* G	Overall	0.016; 57.5%	random-effects (D-L)	1.09 (0.92-1.30) 0.321	0.57; 0.587 (-2.84-4.64)
	Caucasion	0.010; 62.3%	random-effects (D-L)	1.08 (0.89-1.31) 0.414	

Note: D-L, the **DerSimonian-Laird** method; M-H, the Mantel-Haenszel method; p_h, p-value of Q-test for heterogeneity test; p_{OR}, p-value of Z-test for OR; p_E, p-value of t-test for Egger's test.

Table 3: Main results of heterogeneity pooled ORs, stratification analysis and Egger's test of the *MTR* gene functional polymorphisms on AD risk in the meta analysis.

inflammatory bowel disease [36], all of which were characterized by hyperhomocysteinemia caused by impaired one-carbon metabolism [37-39].

There is still a long way to go to fully understand the relationship between folate metabolism and AD. Updated meta analysis studies demonstrated that individuals with AD had higher HCY levels than controls; however, a causal relationship between hyperhomocysteinemia and risk of developing AD was not supported [40], and no benefit of folic acid in reducing cognitive decline was observed [41].

The primary analysis demonstrated that the *MTR* gene has been considered as a candidate gene for AD and *MTR* AA genotype was a risk factor of AD [12]. This meta analysis suggested that no association

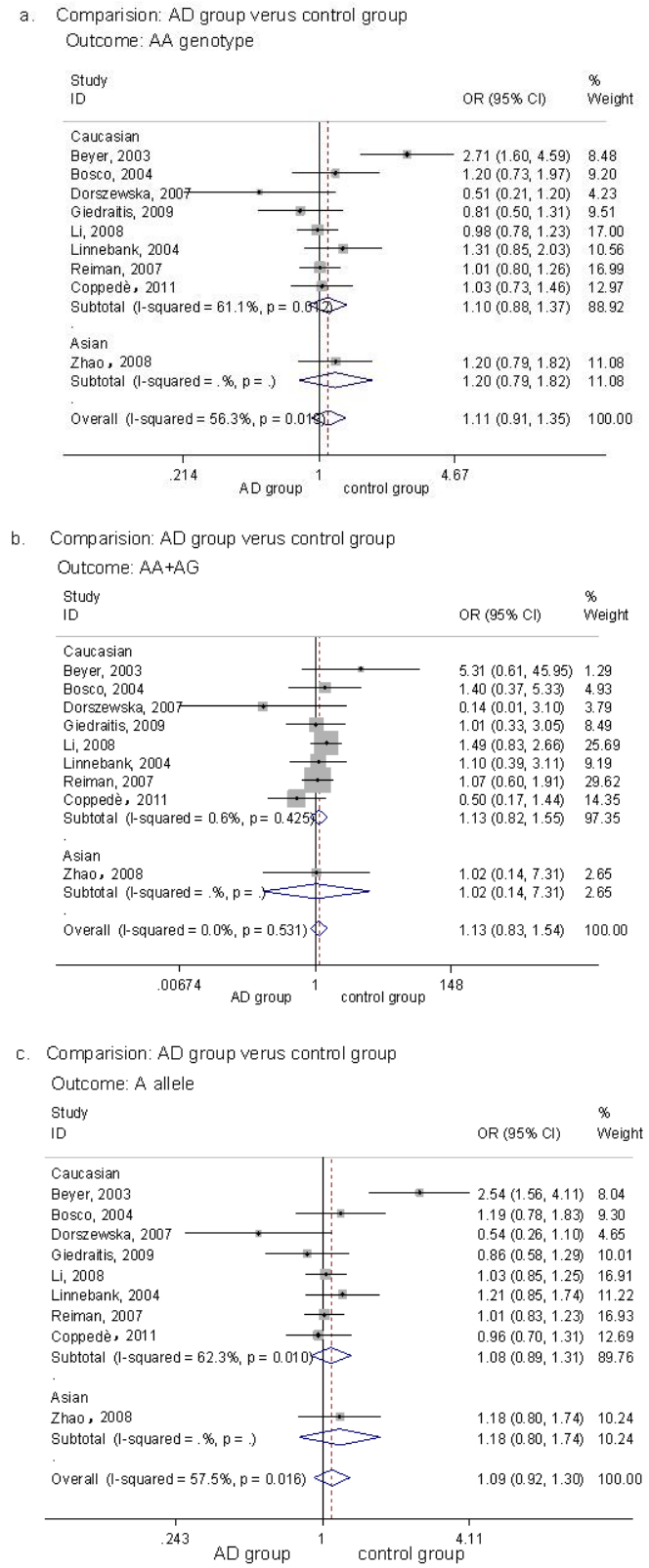

Figure 1: Forest plot of AD risk associated with rs1805087 under contrast of AA *vs* AG+GG (a), AA+AG *vs* GG (b), and A *vs* G(c), respectively, in different ethnicity. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the study specific weight. The diamond represents the pooled OR and 95% CI.

between *MTR* A2756G polymorphism and AD, but the conclusion reached in the present study was based on relatively small numbers of studies and participants.

Genetic power calculator

The overall allele A frequncy was set as 0.83, determined by the included studies. With the overall OR 1.11 in the recessive model, the total genetic power was calculated as 11.6%. For the dominant model, the total genetic power was calculated as 43.7%, with the overall OR 1.13. Neither of the power has the potential to draw a conclusion whether this polymorphism is in association with AD or not (power<90%). So even though our meta analysis suggests that *MTR* A2756G polymorphism is not a genetic determinant of AD, small sample size may be one reason and it could not be ruled out that a true association exists. Whether the *MTR* A2756G polymorphism indeed associated with AD has to be confirmed in additional studies.

Heterogeneity

In the contrast of AA *vs.* AG+GG, large heterogeneity among combined populations (p = 0.019, I^2= 56.3%) and Caucasion subgroup (p=0.012, I^2= 61.1%) study was observed. Large heterogeneity was detected among combined populations (p = 0.016, I^2= 57.5%) and

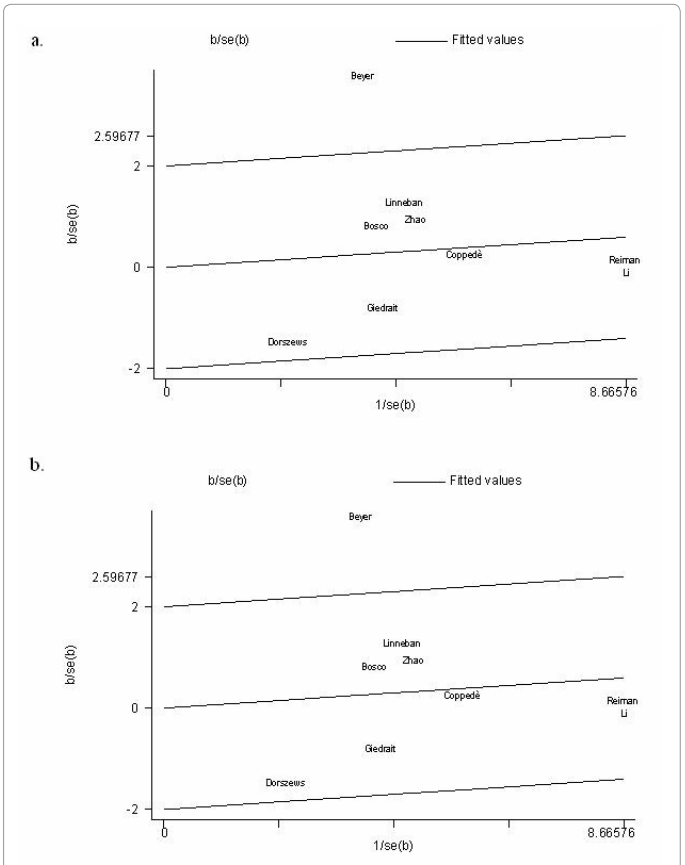

Figure 2: Galbraith plot of associations between *MTR* A2756G polymorphisms and AD risk, produced by first dividing each estimate by its standard error (se) to generate z-statistics (b/se(b)), which is then plotted versus 1/se(b) for each of studies. Inner line represents pooled effect and outer lines represent 95% limits. Each author represents the respective study included in the meta analysis (shown in Table 1) for the indicated association by AA *vs.* AG+GG (a) and A *vs.* G (b).

Caucasion subgroup (p = 0.010, I^2= 62.3%) in the contrast of A *vs.* G. By contrast, no heterogeneity among studies was observed in the contrast of AA+AG *vs.* GG among combined populations (p = 0.531, I^2= 0%) and Caucasion subgroup (p = 0.425, I^2= 0.6%, Table 3).

The AA *vs.* AG+GG and A *vs.* G results showed large heterogeneity among combined populations and Caucasion subgroup studies in this meta analysis. Through stratified analyses, the heterogeneity of the subgroup didn't reduce.

The study of Beyer and co-workers in 2003 [12] was considered as the main cause of heterogeneity in both contrasts as shown in the galbraith plot for heterogeneity (Figures 2a and 2b). After exclusion of this study, the heterogeneity no longer existed, but still reached a negative association (data not shown).

We explored potential sources of heterogeneity in following aspects: (1) Diagnosis of AD. The most frequently used diagnostic criteria for AD are NINCDS-ADRDA, DSM-IV and International Classification of disease-10 (ICD-10). In the included studies, AD was diagnosed by different criteria. These different criteria may result in an inconsistent diagnosis of AD. (2) Genotyping methods. Depending on the center, a broad range panel of technologies were used to genotype the rs1805087 polymorphism, such as restriction fragment length polymorphism (RFLP), genome-wide association study (GWAS), high and ultra-high throughput genotyping, et al. The heterogeneity may not be caused by ethnicity, because it can be found that the heterogeneity doesn't reduce in the subgroup analyses stratified by racial descents.

In the study of Beyer and co-workers in 2003 [12], patients were sporadic AD with clinical diagnosis of probable AD according to the DSM-IV and NINCDS-ADRDA criteria and without a first-degree relative with either AD or progressive memory loss. And the method used for genotyping was RFLP, based on HaeIII-digested PCR. Therefore, it can be presumed that the heterogeneity mainly results from the diagnosis of AD and genotyping methods.

Bias diagnostics

Begg's funnel plot and Egger's test were performed to assess the publication bias of the literature. The shapes of the funnel plot for the contrast of the AA *vs.* AG+GG seemed approximately symmetrical (Figure 3), and Egger's test did not show any evidence of publication bias (t =0.54; p = 0.609; 95%CI -2.54 to 4.11). So did the contrast of the AA+AG *vs.* GG (t =0.77; p = 0.464) and the contrast of A *vs.* G (t =0.57; p = 0.587), as shown in Table 3.

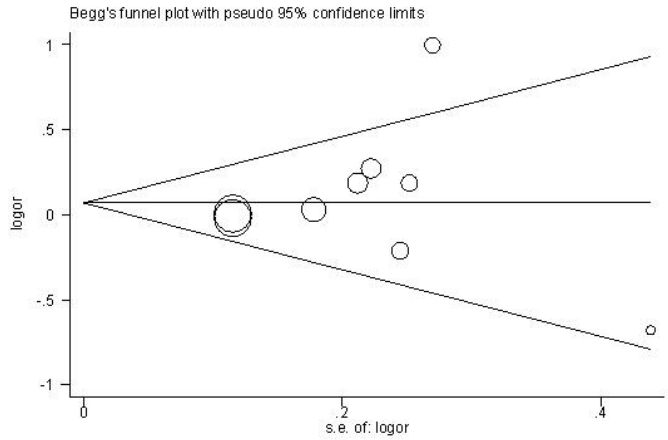

Figure 3: Begg's funnel plot of publication bias in *MTR* A2756G polymorphism studies. Log OR is plotted versus standard error for each of studies in this meta analysis. Each circle represents a separate study for the indicated association by AA *vs* AG+GG.

The result for publication bias was not statistically significant. But publication bias may exist, because only published studies were included in this meta analysis.

Conclusion

In summary, this meta analysis can't prove that the rs1805087 in *MTR* gene is associated with the risk of AD. But small sample size may be one reason and it could not be ruled out that a true association exists. More well- designed studies with larger sample size are warranted to validate these findings.

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